Data Evaluation Record on the Toxicity of Hoe 064619 Technical (Metabolite of Glufosinate-ammonium) to the Green Alga, *Scenedesmus subspicatus*

EPA MRID Number 48444811

Data Requirement:

EPA DP Barcode	345709
EPA MRID	48444811
EPA Guideline	850.5400

Test material: **Hoe 064619 Technical** **Purity:** 98.1%
Common name
Chemical name: IUPAC: 2-methylphosphinico-acetic acid
CAS name:
CAS No.:
Synonyms: MPA

Primary Reviewer: Moncie Wright **Signature:**
Staff Scientist, Cambridge Environmental Inc. **Date:** 7/13/11

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental Inc. **Date:** 10/18/11

Primary Reviewer: Catherine Aubee **Signature:**
US EPA/OPP/EFED/ERBIV **Date:** 30 March 2012

EPA PC Code 128850

Date Evaluation Completed: 30 March 2012

CITATION: Heusel, R. 1993. 2-Methylphosphinico-acetic acid, - substance, technical (Hoe 064619 00 ZC98 0001): Effect to *Scenedesmus subspicatus* (green algae) in a growth inhibition test (method OECD). Unpublished study performed and sponsored by Hoechst AG, Frankfurt am Main, Germany. Study completed February 11, 1993.

EXECUTIVE SUMMARY:

The 72-hour toxicity study evaluated the effects of **Hoe 064619 Technical** (also known as MPA, 98.1% purity), a metabolite of the herbicide glufosinate-ammonium, on cultures of the green alga (*Scenedesmus subspicatus*). Nominal test concentrations were 0 (negative control), 9.8, 17.7, 31.4, 54.9, 98.1, and 177 mg MPA/L (adjusted for purity) under static conditions. Measured concentrations were only determined for a low (9.8 mg MPA/L), middle (54.9 mg MPA/L), and high concentration (177 mg MPA/L). Recoveries in these samples ranged from 97 to 104% of nominal concentrations. As a result, toxicity values in this study calculated by the reviewer were based on a combination of the corrected nominal concentrations and the available measured concentrations.

The most sensitive endpoint was biomass, with NOAEC and EC_{50} values of 17.7 and 53 mg MPA/L, respectively. The percent growth inhibition of cell density in the treated algal culture as compared to the control ranged from -2 to 79%.

In the 31.4 mg MPA/L test level and above, some algal cells were enlarged. The study author reported that the frequency of enlarged cells was treatment-related and concentration-responsive.

This toxicity study is scientifically sound and is classified as **supplemental**; the test does not satisfy OCSPP guideline 850.5400 because pH values were not within the recommended range, the test duration was 72 hours instead of 96 hours, algae in controls had not reached the logarithmic growth phase by test termination, and the light intensity was much higher than what is recommended. These deviations may affect the nature of the growth curve and the concentration-response, thereby limiting the utility of the information in EPA risk assessment.

Results Synopsis

Test Organism: *Scenedesmus subspicatus*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density
EC_{50}: 65 mg MPA/L 95% C.I.: 44-96 mg MPA/L
NOAEC: 17.7 mg MPA/L
Slope: 1.88 ± 0.375

Biomass
EC_{50}: 53 mg MPA/L 95% C.I.: 37-77mg MPA/L
NOAEC: 17.7 mg MPA/L
Slope: 1.98 ± 0.336

Growth rate
EC_{50}: >177 mg MPA/L 95% C.I.: N/A
NOAEC: 17.7 mg MPA/L
Slope: N/A

Endpoint(s) Effected: Cell density, biomass, and growth rate

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test procedure followed the guidelines of the Organization for Economic Cooperation and Development (OECD), Guideline No. 201: Alga, Growth Inhibition Test (1984). The study methods and results were evaluated according to U.S. EPA OPPTS 850.5400: Algal Toxicity, Tiers I and II and OECD No. 201, and differences and/or similarities were described. The following deficiencies and deviations from OPPTS 850.5400 and OECD 201 were noted:

1. The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

2. Analytical verification was not performed for all test concentration levels; OPPTS guidelines suggest that the concentration of the test material in the test vessels should be determined at the beginning and end of the test. OECD guidelines also recommend verifying the test concentrations at the beginning of the test and also verifying that those concentrations have been maintained during the test.

3. The test temperature ranged from 25.6 to 26.6°C; while the species tested is not included in either OPPTS or OECD guidelines, this temperature is still higher than recommended for similar algal species (OPPTS: 24°C and OECD: 21-24°C).

4. The physico-chemical properties of the test material were not reported; OECD guidelines suggest that this information be reported. OPPTS guidelines do not address this topic.

5. The pH of the control ranged from 7.9 to 10.0 and in the test solutions ranged from 7.1 to 10.2; OPPTS guidelines suggest a pH of 7.5 ± 0.2 for similar algal species. Additionally, OECD guidelines suggest that the control pH not vary by more than 1.5 units.

6. Neither the LOQ nor the LOD for the HPLC method were reported.

The deviations do not substantively affect the scientific soundness of the study but may limit interpretation of the results.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study author did not report which GLP standards were adhered to.

A. MATERIALS:

1. Test material **Hoe 064619**

Description: White crystalline powder

Lot No./Batch No. : Not reported

Purity: 98.1%

Stability of compound under test conditions: Analytical verification was only performed for the low, high middle, and highest test level solutions. At time 0, recoveries ranged from 97 to 103% of nominal concentrations. At 72 hours (test termination), recoveries ranged from 97 to 104% of nominal. The test material appeared to be stable under the test conditions.
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Not reported.

Physicochemical properties of Hoe 064619.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Green algae; *Scenedesmus subspicatus* CHODAT

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: 86.81 SAG
Source: In-house cultures originally obtained from the Collection of Algal Cultures, Institute of Plant Physiology, University of Gottingen, Gottingen, Germany
Age of inoculum: 3 days
Method of cultivation: Algae were cultivated in nutrient medium

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A range-finding study was conducted with a control and nominal concentrations of 0.1, 0.32, 1, 3.2, and 10 mg MPA/L. After 72 hours, inhibitions in cell density were 5, 6, 6, 1, and 5%, respectively. Inhibitions in biomass were a maximum of 5%, and inhibitions in growth rate were a maximum of 1%.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Same as test (dilution water, temperature, agitation, photoperiod, and light intensity) Not reported	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
<u>Test system</u> Static/static renewal Renewal rate for static renewal	 Static N/A	 *EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	The test vessels were placed in a waterbath positioned on an electric shaker	
Duration of the test	72 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*

Parameter	Details	Remarks ***Criteria***
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 300 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	 7.0-8.4 8.0-10.2 Yes $NaHCO_3$ N/A	Control pH: 7.9-10.0 *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Deionized water 8.0 N/A Filtered by ultrafiltration, ion exchange, and a charcoal unit Not reported Not reported Not reported Not reported Not reported	The deionized water was used to create reagent grade water that was used to prepare the algal medium. *EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	The test material (0.1 mg) was dissolved in nutrient medium to create a primary stock solution. The solution was shaken well and defined amounts were pipetted proportionally into the test flasks. The flasks were then filled up to 250-650 mL with nutrient medium. Pre-culture (1.71-3.99 mL) was added to the vessels, which were then filled up to 300-700 mL with nutrient medium.	
Aeration or agitation	Agitation; 100 rpm	
Initial cells density	1 x 10^4 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Treatments:	6 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal (unadjusted for purity): Nominal (adjusted for purity): Measured:	0 (negative control), 10, 18, 32, 56, 100, and 180 mg MPA/L 0 (negative control), 9.8, 17.7, 31.4, 54.9, 98.1, and 177 mg MPA/L 0 (negative control), 10.2 (low), 53.5 (high medium), and 172 (high) mg MPA/L *The LOQ was not reported.	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Samples from low, high medium, and high test levels and the control were analyzed via HPLC with UV detection (216 nm). Fortification samples were analyzed concurrently.	

Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	25.6-26.6°C Continuous 180 ± 12 µE.m^{-2}·s^{-1} White spectrum fluorescent lamps of universal white-type L25	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25ºC maintained at ± 2ºC and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	- Cell density - Biomass - Growth rate	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks Criteria
Measurement technique for cell density and other end points	Cell density was determined using Thoma counting chambers (Schreck, Hofheim, Germany) and a microscope (Zeiss, Oberkochen, Germany). The study author did not report how biomass and growth rate values were calculated.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	None.	
Indicate whether there was an exponential growth in the control	Yes; cell density was 192 x 10^4 cells/mL at 72 hours.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

At 72 hours, cell density in the negative control averaged 192 x 10^4 cells/mL, which yielded inhibitions of -2, -2, 57, 27, 63, and 79% as compared to the control. An EC_{50} value was not calculated for this endpoint.

At 72 hours, biomass in the negative control averaged 3680 x 10^4 cells/mL*h, yielding inhibitions of -4, -3, 59, 36, 71, and 83% as compared to the control. The 72-hour EC_{50} value was 69 mg MPA/L.

At 72 hours, the growth rate in the negative control averaged 0.073 hours-1, yielding inhibitions of 0, 0, 17, 6, 19, and 31%. The 72-hour EC_{50} value could not be calculated due to a lack of an effect of ≥50%.

The overall NOAEC value, based on the level at which no significant growth inhibition and no cell deformation was observed, was 18 mg MPA/L.

In the 32 mg MPA/L test level and above, some algal cells were enlarged. The study author reported that the frequency of enlarged cells was dose related.

Table 3: Effect of Hoe 064619 Technical on algal growth of *Scenedesmus subspicatus*.

Treatment Nominal mg MPA/L	**Initial cell Density (x 10^4 cells/mL)**	**Cell density (x 10^4 cells/mL) at**			
		24 hours	**48 hours**	**72 hours**	
				cell count	**% inhibition**
Negative control	1.0	8.4	51.5	191.8	N/A
9.8	1.0	8.7	55.8	194.7	-2
17.7	1.0	8.3	55.2	195.1	-2
31.4	1.0	0.73	23.6	83.3	57
54.9	1.0	3.4	27.0	140.8	27
98.1	1.0	2.2	9.5	71.6	63
177	1.0	1.6	7.1	39.6	79
Reference chemical (if used)	N/A				

Table 4: Effect of Hoe 064619 Technical on algal growth (*Scenedesmus subspicatus*).

Treatment Nominal mg MPA/L	Initial Cell Density ($x10^4$ cells/mL)	Mean Growth Rate (hours^{-1})		Mean Biomass ($x\ 10^4$ cells/mL*h)	
		0-72 Hours	Percent Inhibition	0-72 hours	Percent Inhibition
Negative control	1.0	0.073	N/A	3680.4	N/A
9.8	1.0	0.0732	0	3824.8	-4
17.7	1.0	0.0732	0	3804.8	-3
31.4	1.0	0.0605	17	1523.2	59
54.9	1.0	0.0687	6	2359.2	36
98.1	1.0	0.0592	19	1080.8	71
177	1.0	0.0507	31	624.8	83

Table 5: Statistical endpoint values.

Statistical Endpoint	Cell density	Growth rate	Biomass
NOAEC or EC_{05} (mg MPA/L)	18	18	18
EC_{50} (mg MPA/L) (95% C.I.)	ND	N/A	69 (18-100)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A		

B. REPORTED STATISTICS:

The EC_{50} value for growth rate could not be determined due to a lack of an inhibitory effect of ≥50%. The cell density data were not analyzed. The biomass data were analyzed using computer programs (Scientific Software, 1986 and Stephan et al., 1978). The study author selected the binomial method, moving average, or probit method for the determination of the EC50 value depending on which method provided the narrowest confidence limits. The NOAEC was determined using a multiple t-test in SAS via Duncan's test. Nominal concentrations were used for analysis.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer tested cell density, biomass, and growth rate replicate data for normality using the Chi-square and Shapiro Wilk's tests and for homogeneity of variance using Levene's test in Toxstat 3.5. All endpoint data met the assumptions of ANOVA, and were thus analyzed using the Bonferroni t-test and Williams' tests to determine the NOAEC. The ECx values (with 95% C.I.) and probit slope were determined using the probit analysis in Nuthatch.

All toxicity values were determined using a combination of the available measured concentrations and the 72-hour nominal concentrations adjusted for the percent purity (for the concentrations that were not analytically determined). Cell density values were entered into Toxstat 3.5 as an abbreviated value, representing the value x 10^4.

Cell density
EC_{50}: 65 mg MPA/L 95% C.I.: 44-96 mg MPA/L
NOAEC: 17.7 mg MPA/L
Slope: 1.88 ± 0.375

Biomass
EC_{50}: 53 mg MPA/L 95% C.I.: 37-77mg MPA/L
NOAEC: 17.7 mg MPA/L
Slope: 1.98 ± 0.336

Growth rate
EC_{50}: >177 mg MPA/L 95% C.I.: N/A
NOAEC: 17.7 mg MPA/L
Slope: N/A

D. STUDY DEFICIENCIES:

The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

Analytical verification was not performed for all test levels.

E. REVIEWER'S COMMENTS:

The reviewer's and the study author's NOAEC values were in general agreement when taking into consideration the difference between nominal concentrations that were adjusted for purity versus nominal concentrations that were not corrected for the percent purity, as well as the reviewer's use of the three available measured concentrations. The reviewer's EC_{50} value for biomass was much lower than the study author's value. Additionally, the reviewer obtained EC_{50} values for cell density and growth rate, while the study author did not attempt determinations. The reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The range-finding test was conducted from June 9 to 12, 1992.

The definitive test was initiated June 23, 1992, and was terminated June 26, 1992.

F. CONCLUSIONS:

This toxicity study is scientifically sound and is classified as **supplemental**; the test does not satisfy OCSPP guideline 850.5400 because pH values were not within the recommended range, the test duration was 72 hours instead of 96 hours, algae in controls had not reached the logarithmic growth phase by test termination, and the light intensity was much higher than what is recommended. These deviations may affect the nature of the growth curve and the concentration-response, thereby limiting the utility of the information in EPA risk assessment. The most sensitive endpoint was biomass, with NOAEC and EC_{50} values of 17.7 and 53 mg MPA/L, respectively.

Cell density
EC_{50}: 65 mg MPA/L 95% C.I.: 44-96 mg MPA/L
NOAEC: 17.7 mg MPA/L
Slope: 1.88 ± 0.375

Biomass
EC_{50}: 53 mg MPA/L 95% C.I.: 37-77mg MPA/L
NOAEC: 17.7 mg MPA/L
Slope: 1.98 ± 0.336

Growth rate
EC_{50}: >177 mg MPA/L 95% C.I.: N/A
NOAEC: 17.7 mg MPA/L
Slope: N/A

Endpoint(s) Effected: Cell density, biomass, and growth rate

III. REFERENCES:

Organization for Economic Cooperation and Development, 1984. OECD Guidelines for Testing of Chemicals. Guideline No. 201: Alga, Growth Inhibition Test. 07 June 1984.

Anhang (zu §19a, Absatz 1) des Chemikaliengestzes vom 01.August 1990 im Wortlaut einer Bekanntmachung des Bundesministers fur Umwelt, Naturschutz und Reaktorsicherheit (Prof. Dr. Topfer) vom 14. Marz 1990 (veroffentlicht im Bundesgesetzblatt, 22. Marz 1990).

Bundesminister fur Jugend, Familie und Gesundheit, 04.02.1983. Bekanntmachung der OECD-Grundsatze der Guten Laborpraxis (GLP). Translation of OECD Principles of Good Laboratory Practice, OECD Guidelines for Testing of Chemicals, OECD, Paris, 1981), Bundesanzeiger Nummer 42a vom 02. Marz 1983.

Kuhl, A. and Lorenzen, H., 1964. Handling and Culturing of Chlorella, in Methods of Cell Physiology, Vol. I, New York – London, pp. 159-187.

U.S. Environmental Protection Agency (EPA), 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants. U.S. Department of Commerce, PB83-153940, 27 October 1982.

U.S. Environmental Protection Agency (EPA), 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR Part 792). Fed. Reg.,Vol. 48, No. 230, Nov. 23, 1983, pp. 53922-53944.

Stephan, C.E., K.A. Bush, R. Smith, J. Burke, R.W. Andrew, 1978. A Computer Program for Calculating an LC_{50}. U.S. Environmental Protection Agency, Duluth, MN. Pre-publication manuscript, August 1978.

Jandel Scientific, 1987. A Computer Program for Plotting the Growth Curves and the Concentration-Effect Relationship, SigmaPlot, version 3.1.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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  Title:  Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
File:             4811c             Transform:             NO TRANSFORMATION

                      Chi-Square Test for Normality
-------------------------------------------------------------------------------

                     Actual and Expected Frequencies

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
             -----     -------------     -----------    -----------    -----
EXPECTED    1.6080        5.8080            9.1680         5.8080     1.6080
OBSERVED     1             7                 7              9          0

-------------------------------------------------------------------------------

     Chi-Square =   4.3495             (p-value = 0.3608)

     Critical Chi-Square   = 13.277  (alpha = 0.01 , df = 4)
                           =  9.488  (alpha = 0.05 , df = 4)

-------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
File:             4811c             Transform:             NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
-------------------------------------------------------------------------------

     D = 4470.8333
     W =   0.9533

     Critical W  = 0.8840 (alpha = 0.01 , N = 24)
              W  = 0.9160 (alpha = 0.05 , N = 24)

-------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
File:             4811c             Transform:             NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
-------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
-------------------------------------------------------------------------------
   Between             6            940.7917         156.7986       1.4404

   Within (Error)     17           1850.5867         108.8580
-------------------------------------------------------------------------------
   Total              23           2791.3783
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------------------------------------------------------------------------------
                                                          (p-value = 0.2568)

   Critical F  = 4.1015  (alpha = 0.01, df = 6,17)
               = 2.6987  (alpha = 0.05, df = 6,17)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)


Title:  Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
File:             4811c             Transform:             NO TRANSFORMATION

                             ANOVA Table
------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
------------------------------------------------------------------------------
   Between             6          88048.4717       14674.7453      55.7996

   Within (Error)     17           4470.8333         262.9902
------------------------------------------------------------------------------
   Total              23          92519.3050
------------------------------------------------------------------------------
                                                          (p-value = 0.0000)

   Critical F  = 4.1015  (alpha = 0.01, df = 6,17)
               = 2.6987  (alpha = 0.05, df = 6,17)

   Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)


Title:  Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
File:             4811c             Transform:             NO TRANSFORMATION

     Bonferroni t-Test   -   TABLE 1 OF 2            Ho: Control<Treatment
------------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN           SIG
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    t STAT  0.05
----- --------------------    -----------    ------------------  ------  ---
  1            Neg control    191.7667           191.7667
  2                    9.8    194.6667           194.6667       -0.2529
  3                   17.7    195.1333           195.1333       -0.2936
  4                   31.4     83.2667            83.2667        9.4618  *
  5                   54.9    140.8000           140.8000        4.4446  *
  6                   98.1     71.6000            71.6000       10.4792  *
  7                    177     39.6000            39.6000       13.2698  *
------------------------------------------------------------------------------
Bonferroni t critical value = 2.6550   (1 Tailed, alpha = 0.05, df = 6,17)


Title:  Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
File:             4811c             Transform:             NO TRANSFORMATION

     Bonferroni t-Test   -   TABLE 2 OF 2            Ho: Control<Treatment
------------------------------------------------------------------------------
                            NUM OF     MIN SIG DIFF     % OF     DIFFERENCE
GROUP    IDENTIFICATION      REPS    (IN ORIG. UNITS)  CONTROL  FROM CONTROL
----- --------------------  -------  ----------------  -------  ------------
  1            Neg control     6
```

2	9.8	3	30.4452	15.9	-2.9000
3	17.7	3	30.4452	15.9	-3.3667
4	31.4	3	30.4452	15.9	108.5000
5	54.9	3	30.4452	15.9	50.9667
6	98.1	3	30.4452	15.9	120.1667
7	177	3	30.4452	15.9	152.1667

Title: Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
File: 4811c Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	191.7667	191.7667	193.3333
2	9.8	3	194.6667	194.6667	193.3333
3	17.7	3	195.1333	195.1333	193.3333
4	31.4	3	83.2667	83.2667	112.0333
5	54.9	3	140.8000	140.8000	112.0333
6	98.1	3	71.6000	71.6000	71.6000
7	177	3	39.6000	39.6000	39.6000

Title: Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
File: 4811c Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	191.7667				
9.8	193.3333	-0.1366		1.7400	k= 1, v=17
17.7	193.3333	-0.1366		1.8200	k= 2, v=17
31.4	112.0333	6.9532	*	1.8500	k= 3, v=17
54.9	112.0333	6.9532	*	1.8700	k= 4, v=17
98.1	71.6000	10.4792	*	1.8700	k= 5, v=17
177	39.6000	13.2698	*	1.8800	k= 6, v=17

s = 16.2170

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	8.7	2.9	26.	0.23	0.33
EC10	14.	5.4	34.	0.19	0.40
EC25	28.	15.	54.	0.13	0.52
EC50	65.	44.	96.	0.081	0.68

Slope = 1.88 Std.Err. = 0.375

```
!!!Poor fit: p < 0.001 based on DF=     4.00     17.0
-------------------------------------------------------------------------
4811C : Hoe 064619 & S. subspicatus 72-hr cell density; mg MPA/L
-------------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
-------------------------------------------------------------------------
     Dose     #Reps.     Obs.     Pred.      Obs.     Pred.   %Change
                         Mean     Mean     -Pred.   %Control

     0.00      6.00      192.      196.     -4.31      100.      0.00
     10.2      3.00      195.      183.      11.3      93.5      6.50
     17.7      3.00      195.      168.      27.3      85.6      14.4
     31.4      3.00      83.3      142.     -58.7      72.4      27.6
     53.5      3.00      141.      110.      30.4      56.3      43.7
     98.1      3.00      71.6      72.1    -0.519      36.8      63.2
     172.      3.00      39.6      41.7     -2.11      21.3      78.7

!!!Warning: EC5 not bracketed by doses evaluated.

  Title:  Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
  File:             4811b             Transform:             NO TRANSFORMATION

                        Chi-Square Test for Normality
  -------------------------------------------------------------------------

                       Actual and Expected Frequencies

  INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
               -----     -------------     -----------    -----------    -----
  EXPECTED    1.6080        5.8080            9.1680         5.8080     1.6080
  OBSERVED     0             9                 6              9          0

  -------------------------------------------------------------------------

       Chi-Square =   7.8193             (p-value = 0.0984)

       Critical Chi-Square   = 13.277  (alpha = 0.01 , df = 4)
                             =  9.488  (alpha = 0.05 , df = 4)

  -------------------------------------------------------------------------

  Data PASS normality test (alpha = 0.01). Continue analysis.


  Title:  Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
  File:             4811b             Transform:             NO TRANSFORMATION

                    Shapiro - Wilk's Test for Normality
  -------------------------------------------------------------------------

       D = 1416054.7200
       W =   0.9580

       Critical W  = 0.8840 (alpha = 0.01 , N = 24)
                W  = 0.9160 (alpha = 0.05 , N = 24)

  -------------------------------------------------------------------------
```

```
Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
File:             4811b             Transform:              NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
-------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
-------------------------------------------------------------------------------
   Between             6         385899.8400       64316.6400       2.4360

   Within (Error)     17         448835.5200       26402.0894
-------------------------------------------------------------------------------
   Total              23         834735.3600
-------------------------------------------------------------------------------
                                                              (p-value = 0.0695)

   Critical F  = 4.1015  (alpha = 0.01, df = 6,17)
               = 2.6987  (alpha = 0.05, df = 6,17)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)


Title:  Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
File:             4811b             Transform:              NO TRANSFORMATION

                               ANOVA Table
-------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
-------------------------------------------------------------------------------
   Between             6       38120803.9202     6353467.3200      76.2746

   Within (Error)     17        1416054.7200       83297.3365
-------------------------------------------------------------------------------
   Total              23       39536858.6401
-------------------------------------------------------------------------------
                                                              (p-value = 0.0000)

   Critical F  = 4.1015  (alpha = 0.01, df = 6,17)
               = 2.6987  (alpha = 0.05, df = 6,17)

   Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)


Title:  Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
File:             4811b             Transform:              NO TRANSFORMATION

     Bonferroni t-Test   -   TABLE 1 OF 2            Ho: Control<Treatment
-------------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN          SIG
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    t STAT  0.05
----- --------------------    -----------    ------------------  ------  ---
  1            Neg control   3680.4000          3680.4000
  2                    9.8   3824.8000          3824.8000       -0.7076
```

3	17.7	3804.8000	3804.8000	-0.6096	
4	31.4	1523.2000	1523.2000	10.5704	*
5	54.9	2359.2000	2359.2000	6.4739	*
6	98.1	1080.8000	1080.8000	12.7381	*
7	177	624.8000	624.8000	14.9726	*

Bonferroni t critical value = 2.6550 (1 Tailed, alpha = 0.05, df = 6,17)

Title: Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
File: 4811b Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	6			
2	9.8	3	541.8316	14.7	-144.4000
3	17.7	3	541.8316	14.7	-124.4000
4	31.4	3	541.8316	14.7	2157.2000
5	54.9	3	541.8316	14.7	1321.2000
6	98.1	3	541.8316	14.7	2599.6000
7	177	3	541.8316	14.7	3055.6000

Title: Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
File: 4811b Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	3680.4000	3680.4000	3747.6000
2	9.8	3	3824.8000	3824.8000	3747.6000
3	17.7	3	3804.8000	3804.8000	3747.6000
4	31.4	3	1523.2000	1523.2000	1941.2000
5	54.9	3	2359.2000	2359.2000	1941.2000
6	98.1	3	1080.8000	1080.8000	1080.8000
7	177	3	624.8000	624.8000	624.8000

Title: Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
File: 4811b Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	3680.4000				
9.8	3747.6000	-0.3293		1.7400	k= 1, v=17
17.7	3747.6000	-0.3293		1.8200	k= 2, v=17
31.4	1941.2000	8.5221	*	1.8500	k= 3, v=17

```
                 54.9  1941.2000    8.5221      *     1.8700       k= 4, v=17
                 98.1  1080.8000   12.7381      *     1.8700       k= 5, v=17
                  177   624.8000   14.9726      *     1.8800       k= 6, v=17
  ------------------------------------------------------------------------------
  s = 288.6128

   WARNING: Procedure has used isotonized means which differ from original
            (transformed) means.

Estimates of EC%
--------------------------------------------------------------------------
Parameter   Estimate     95% Bounds        Std.Err.  Lower Bound
                       Lower     Upper               /Estimate
EC5             7.9       3.1       20.      0.20      0.39
EC10            12.       5.4       27.      0.17      0.45
EC25            24.       14.       43.      0.12      0.56
EC50            53.       37.       77.     0.075      0.70

         Slope =     1.98  Std.Err. =    0.336


!!!Poor fit: p < 0.001 based on DF=     4.00     17.0
--------------------------------------------------------------------------
4811B : Hoe 064619 & S. subspicatus 72-hr biomass; mg MPA/L
--------------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
--------------------------------------------------------------------------
     Dose     #Reps.     Obs.      Pred.      Obs.      Pred.    %Change
                         Mean      Mean     -Pred.    %Control

     0.00       6.00  3.68e+03  3.82e+03     -141.      100.       0.00
     10.2       3.00  3.82e+03  3.53e+03      298.      92.3       7.70
     17.7       3.00  3.80e+03  3.17e+03      636.      82.9       17.1
     31.4       3.00  1.52e+03  2.58e+03 -1.06e+03      67.6       32.4
     53.5       3.00  2.36e+03  1.91e+03      451.      49.9       50.1
     98.1       3.00  1.08e+03  1.15e+03     -66.8      30.0       70.0
     172.       3.00       625.      600.      24.9      15.7       84.3

!!!Warning: EC5 not bracketed by doses evaluated.

  Title:  Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L
  File:             4811g             Transform:             NO TRANSFORMATION

                        Chi-Square Test for Normality
  ------------------------------------------------------------------------------

                       Actual and Expected Frequencies

  INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
               -----     -------------     -----------    -----------    -----
  EXPECTED    1.6080        5.8080            9.1680         5.8080     1.6080
  OBSERVED     1             7                 7              9          0

  ------------------------------------------------------------------------------

       Chi-Square =   4.3495             (p-value = 0.3608)

       Critical Chi-Square   = 13.277  (alpha = 0.01 , df = 4)
                             =  9.488  (alpha = 0.05 , df = 4)

  ------------------------------------------------------------------------------
```

```
Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L
File:             4811g             Transform:             NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
------------------------------------------------------------------------------

     D =   0.0001
     W =   0.8884

     Critical W  = 0.8840 (alpha = 0.01 , N = 24)
              W  = 0.9160 (alpha = 0.05 , N = 24)

------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L
File:             4811g             Transform:             NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                             ANOVA Table
------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
------------------------------------------------------------------------------
   Between             6              0.0000           0.0000       1.7497

   Within (Error)     17              0.0001           0.0000
------------------------------------------------------------------------------
   Total              23              0.0001
------------------------------------------------------------------------------
                                                        (p-value = 0.1700)

   Critical F  = 4.1015  (alpha = 0.01, df = 6,17)
               = 2.6987  (alpha = 0.05, df = 6,17)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)


Title:  Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L
File:             4811g             Transform:             NO TRANSFORMATION

                             ANOVA Table
------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
------------------------------------------------------------------------------
   Between             6              0.0016           0.0003      33.0742

   Within (Error)     17              0.0001           0.0000
------------------------------------------------------------------------------
   Total              23              0.0017
------------------------------------------------------------------------------
```

(p-value = 0.0000)

Critical F = 4.1015 (alpha = 0.01, df = 6,17)
= 2.6987 (alpha = 0.05, df = 6,17)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L
File: 4811g Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05
1	Neg control	0.0730	0.0730		
2	9.8	0.0732	0.0732	-0.1349	
3	17.7	0.0732	0.0732	-0.1349	
4	31.4	0.0605	0.0605	6.3237	*
5	54.9	0.0687	0.0687	2.1585	
6	98.1	0.0592	0.0592	6.9477	*
7	177	0.0507	0.0507	11.2478	*

Bonferroni t critical value = 2.6550 (1 Tailed, alpha = 0.05, df = 6,17)

Title: Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L
File: 4811g Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	6			
2	9.8	3	0.0052	7.2	-0.0003
3	17.7	3	0.0052	7.2	-0.0003
4	31.4	3	0.0052	7.2	0.0125
5	54.9	3	0.0052	7.2	0.0043
6	98.1	3	0.0052	7.2	0.0137
7	177	3	0.0052	7.2	0.0222

Title: Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L
File: 4811g Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	0.0730	0.0730	0.0731
2	9.8	3	0.0732	0.0732	0.0731
3	17.7	3	0.0732	0.0732	0.0731
4	31.4	3	0.0605	0.0605	0.0646

5	54.9	3	0.0687	0.0687	0.0646
6	98.1	3	0.0592	0.0592	0.0592
7	177	3	0.0507	0.0507	0.0507

Title: Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L
File: 4811g Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	0.0730				
9.8	0.0731	-0.0675		1.7400	k= 1, v=17
17.7	0.0731	-0.0675		1.8200	k= 2, v=17
31.4	0.0646	4.2411	*	1.8500	k= 3, v=17
54.9	0.0646	4.2411	*	1.8700	k= 4, v=17
98.1	0.0592	6.9477	*	1.8700	k= 5, v=17
177	0.0507	11.2478	*	1.8800	k= 6, v=17

s = 0.0028

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	24.	9.1	61.	0.20	0.39
EC10	45.	23.	88.	0.14	0.52
EC25	1.3E+02	97.	1.8E+02	0.067	0.73
EC50	4.4E+02	2.4E+02	8.1E+02	0.13	0.55

Slope = 1.29 Std.Err. = 0.300

!!!Poor fit: p = 0.0010 based on DF= 4.0 17.

4811G : Hoe 064619 & S. subspicatus 72-hr growth rate; mg MPA/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	0.0730	0.0732	-0.000205	100.	0.00
10.2	3.00	0.0732	0.0719	0.00132	98.3	1.71
17.7	3.00	0.0732	0.0706	0.00265	96.5	3.54
31.4	3.00	0.0605	0.0681	-0.00768	93.1	6.87
53.5	3.00	0.0687	0.0646	0.00414	88.2	11.8
98.1	3.00	0.0592	0.0586	0.000599	80.1	19.9
172.	3.00	0.0507	0.0514	-0.000662	70.2	29.8

!!!Warning: EC50 not bracketed by doses evaluated.